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A repeatable method for determination of carboxyhemoglobin levels in smokers

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Fourteen male smokers participated in ten afternoon test sessions to determine the daily variation in expired breath carbon monoxide (CO), and whole blood percent carboxyhemoglobin (%COHb), hemoglobin and hematocrit levels. Each individual's test session was conducted at approximately the same time of day to estimate CO-related measures under relatively stable conditions. Subjects smoked *ad libitum* prior to testing. The 'usual brand' cigarette was smoked during the first measurement week (sessions 1–5 held on Monday through Friday) and a research cigarette prototype which primarily heats rather than burns tobacco (TOB-HT) was smoked by 12 of the 14 subjects for 3 weeks prior to and during the second measurement week (sessions 6–9 held on Tuesday through Friday). Following the last 'usual brand' measurement session, subjects completed 21 days of *ad libitum* smoking of the TOB-HT cigarette before starting sessions 6–9 to allow acclimation to the TOB-HT research cigarette. Following session 9, 11 of the 14 subjects continued to smoke the TOB-HT cigarette for an additional 3 weeks and then participated in an additional test session (session 10). The data indicate that daily measurements of afternoon %COHb and expired breath CO values for an individual are reproducible when using this protocol. Carboxyhemoglobin and expired breath CO levels were elevated by 24.4 and 30.6%, respectively, after switching to the TOB-HT cigarette. This increase was not due to an increase in the number of cigarettes consumed since the subjects smoked an average of 21 cigarettes prior to the measurement session when smoking either their 'usual brand' or the TOB-HT cigarette.

Keywords: repeatable method; COHb; smokers

Introduction

The purpose of the present study was to develop a standard measurement protocol that would facilitate the accurate and repeatable determination of %COHb levels in smokers. Cigarette smoke is a known source of CO in smokers, and beginning with the report of Hanson and Hastings,[1] there have been at least 98 studies that have reported various levels of COHb concentration in smokers. Percent COHb concentrations in smokers reported in the literature demonstrate the wide range of reported mean concentrations, with a high value of >18% reported by Lightfoot[2] using only two subjects and a low value of 1.73% (+0.74) reported on 280 subjects by Klesges and Klesges.[3] The heterogeneity of the COHb values reported is not surprising after consideration of the following differences among studies: (1) time of the day that the blood sample was taken; (2) the number of cigarettes smoked prior to sampling; (3) degree of additional CO exposure from occupational or ambient sources; (4) the type of cigarette smoked; (5) the medical condition and demographic category of the subjects; (6) measurement techniques used.

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Inhaled carbon monoxide (CO) readily absorbs through the vasculature of the lungs and binds to hemoglobin (Hb) with an affinity approximately 240 times greater than the affinity of oxygen for Hb.[4] The cherry-red compound formed by the binding of CO with Hb is termed carboxyhemoglobin (COHb).[5] COHb is fully dissociable after termination of CO exposure, and is eliminated almost entirely through the lungs[5] with a half-life of 2 to 6.5 h dependent upon the initial level of COHb and the respiration rate.[6] The normal concentration of COHb in the blood of nonsmoking adults due to endogenous production and ambient exposure usually ranges from about 0.5% to not more than 1%.[7]

The only way to determine the absolute daily 'peak' %COHb level in an inhaling smoker would be to take a measurement shortly after smoking the last cigarette of the day. Since the time when the last cigarette is smoked varies widely for individual subjects, and may occur late at night, a determination after the 'last cigarette' is not feasible as a standard protocol. Therefore, it was decided to take all %COHb measurements in the afternoon. While the afternoon measurement does not give a peak %COHb value, the pharmacokinetic profile of CO absorption shows that a majority of the binding of CO to Hb occurs during the early part of the

exposure period.[8,9] Therefore, afternoon measurements should provide relatively stable values.

A notable feature of our study is that the cigarettes smoked by the subjects prior to sessions 6–10 were research cigarette prototypes which primarily heat rather than burn tobacco (TOB-HT). Published studies on %COHb levels attained after smoking TOB-HT cigarettes are limited. However, Sutherland *et al.*[10] compared the mean expired breath CO level in 20 smokers after switching from tobacco burning cigarettes to an earlier, commercially available TOB-HT cigarette. After the 20 smokers switched to the earlier TOB-HT cigarette for 3 days, there was an increase in expired breath CO levels of 19%.

Methods

Subjects

The subjects were 14 white male smokers, ages 32–45, who smoked full flavor, low-'tar' or ultra-low 'tar' cigarettes as their 'usual brands'. Subjects had no history of diabetes, cardiovascular disease, pulmonary disease or kidney ailments. Each subject reported smoking at least 20 cigarettes per day. After receiving a brief explanation of the experimental protocol, each participant provided informed consent. Subjects appeared for ten afternoon test sessions, each lasting approximately 10 min. All 14 subjects completed sessions 1–5, 12 subjects completed sessions 6–9 and 11 completed session 10. After the completion of session 5, two subjects were dropped from the study because of noncompliance and one subject withdrew after session 9 because of a job transfer. All subjects were company employees and received monetary compensation for their participation at the conclusion of the study.

Cigarettes

During sessions 1–5, the subjects smoked their 'usual brand' of cigarettes. The TOB-HT cigarette was smoked during sessions 6–10. As calculated by the Federal Trade Commission (FTC) method, the ranges of mainstream smoke yields for the 'usual brands' were as follows: nicotine – 0.54 to 1.32 mg/cig.; 'tar' – 5.90 to 17.6 mg/cig.; CO – 8.2 to 17.1 mg/cig. FTC yields for the TOB-HT (test) cigarette were: nicotine – 0.14 mg/cig.; 'tar' – 3.0 mg/cig.; CO – 7.7 mg/cig.

Protocol

Measurement procedures were conducted in the RJR company medical department. Measurement sessions 1–5 were individually scheduled between 2.30 p.m. and 5.00 p.m., Monday through Friday. Measurement sessions 6–9 were individually scheduled between 2.30 p.m. and 5.00 p.m., Tuesday through Friday. Sessions 5 and 6 were separated by 21 days to allow subjects to become acclimated to smoking the test cigarette.

Expired breath CO measurements were made during all ten sessions. To avoid excessive discomfort caused by a daily needle puncture, %COHb, hemoglobin and hematocrit measurements were made only in alternate sessions except for sessions 8 and 9 in which blood was drawn on consecutive days. Venipuncture was performed using a sterile 21 gauge needle inserted into the antecubital vein of the arm. The blood sample was drawn into a 5 ml lavender cap Vacutainer® tube containing EDTA. Percent COHb concentration was determined within 5 min post venipuncture.

Subjects smoked *ad libitum* prior to the measurement sessions. Therefore, the 'number of cigarettes smoked' and the 'number of minutes since the last cigarette' were recorded because these values varied from day-to-day and from subject to subject. The average number of cigarettes smoked prior to a measurement session was 21 for both the 'usual brand' and the test cigarettes.

CO measurements

The expired breath CO concentrations were measured using an ECOLYZER® 2000 CO monitor (National Drager). The subject was instructed to take a deep breath thereby filling his lungs to near capacity, hold the breath for 1 to 2 s, and then to exhale approximately half of the air. The remaining air in the lungs was then blown into a 500 ml Teflon™ bag. The bag was clamped as the air filled the bag and before the subject removed the mouth piece. This method prevented any dilution of the carbon monoxide measurement by outside air.

Percent carboxyhemoglobin concentrations were determined using an IL 482™ CO-Oximeter (Instrumentation Laboratories). The blood sample was tested at least twice to determine the %COHb concentration. A difference of not greater than 0.2% between two consecutive test results was necessary before considering the measurement to be accurate. These two numbers were then averaged for the %COHb value.

Hemoglobin and hematocrit measurements

Blood was taken from the five ml Vacutainer® tube to determine the hemoglobin and hematocrit levels. A microhematocrit capillary tube was filled and spun at 12 000 r.p.m. for 25 min in a Marathon 13K/H centrifuge to determine the hematocrit level. A microcuvette filled with the subject's blood was placed in a HemoCue® hemoglobin analyzer to determine the total hemoglobin level.

Statistical methods

The data were analyzed using analysis of variance. The cigarette variable was treated as a two-level

('usual brand' and the TOB-HT cigarette) fixed effect. The 'subject' and the 'subject by cigarette' interaction terms were treated as random effects. This procedure is roughly equivalent to averaging the data for each subject for each cigarette and then running a paired *t*-test. However, the procedure does differ slightly from a paired *t*-test because some subjects missed one or more sessions creating imbalance in the data set.

The %COHb and the expired breath CO variables were both analyzed in the logarithmic scale. This was done because both variables ranged over a wide span from subject to subject and appeared to suffer from heteroscedasticity, i.e., a difference in variances, in the raw scale. It was assumed that there were no systematic time effects, since it was expected that the 'usual brand' sessions and the TOB-HT sessions would be balanced between the two time periods.

Results

Table 1 shows the group mean values for %COHb, expired breath CO, hemoglobin (grams/deciliter) and % hematocrit for the 12 subjects after smoking both their 'usual brand' and the TOB-HT cigarette. After smoking the 'usual brand', the group mean %COHb value was 8.2%. After switching to the TOB-HT cigarette, the group mean %COHb value rose to 10.2%, a percentage increase of 24.4% compared with 'usual brand'. This increase was statistically significant (P=0.028). Similarly, the mean expired breath CO level was 23.5 p.p.m. after smoking the 'usual brand' and 30.7 p.p.m. after switching to the TOB-HT cigarette. This represented a statistically significant (P=0.014) increase of 30.6% in expired breath CO after switching to the TOB-HT cigarette.

Mean hemoglobin levels increased from 16.2 to 16.6 g/dl, a percentage increase of 2.5%, after switching to the TOB-HT cigarette. This increase was statistically significant (P=0.037). In contrast, the 0.65% increase in mean hematocrit from 46.5 to 46.8% measured after switching to the TOB-HT cigarette was not statistically significant (P=0.368). The normal range for total hemoglobin in adult males is 14 to 18 g/dl. The normal range for hematocrit in adult males is 40 to 54%.[11]

Figure 1 illustrates good consistency in the measurement of %COHb levels for the 14 subjects after smoking their 'usual brand' and the subset of 12 subjects who smoked the TOB-HT cigarette. After smoking their 'usual brand', the average %COHb value among the smokers was 8.2% with a range of 1.4% to 14.5% for the individual readings. After smoking the TOB-HT product, the average %COHb value was 10.2% with a range of 2.5 to 20.7%. The sample day-to-day within subject pooled %COHb standard deviation was 0.94 for the 'usual brand' and 1.37 for the TOB-HT cigarette.

The 'time of day' that the %COHb measurements were taken did not differ statistically between 'usual brand' and the TOB-HT cigarette. On average, the TOB-HT cigarettes were tested 4.5 min later in the day than the 'usual brand' cigarettes. In addition to the 'time of day' measurement, the subjects also reported the 'minutes since their last cigarette' prior to the %COHb measurement. Similarly, there was no statistical difference between 'usual brand' and the TOB-HT cigarette for the amount of time since the last cigarette. The average times since the last cigarette were 16.6 min for the 'usual brand' and 19.6 min for the TOB-HT cigarette. If examined as a potential confounding factor in the analysis, this nominal difference in time since the last cigarette would be predicted to increase the difference in %COHb between the two cigarettes by about 0.1%. This calculation used the standard CFK model and projected the %COHb for a TOB-HT smoker at 16.6 min since the last cigarette. The TOB-HT smoker was at the overall average of 10.2% COHb at 19.6 min since the last cigarette.

Table 1 Group means and comparisons for expired breath CO, %COHb, hemoglobin and hematocrit

	COHb (%)	*Expired breath CO (p.p.m.)*	*Hemoglobin (g/dl)*	*Hematocrit (%)*
Usual brand	8.2	23.5	16.2	46.5
TOB-HT cig.	10.2	30.7	16.6	46.8
P-values	0.028	0.014	0.037	0.368

The group means are computed by averaging the values for each subject and then averaging the subject averages across all subjects *who completed the first nine sessions. Statistical significance* for %COHb and expired breath CO are calculated using the logarithm of the values rather than the raw values

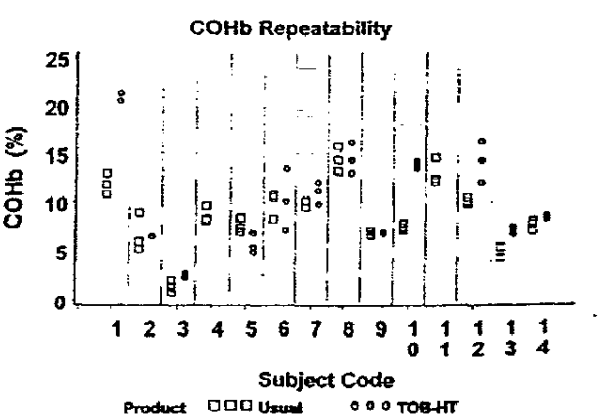

Figure 1 Graph of the repeatability of %COHb measurements for each subject when smoking either their 'usual brand' or the TOB-HT cigarette. Three measurements were taken over 5 days

The consistency of repeated measurements of expired breath CO for the subjects after smoking both their 'usual brand' and the TOB-HT cigarette is shown in Figure 2. The average expired breath CO was 23.5 p.p.m. while smoking the 'usual brand' and 30.7 p.p.m. while smoking the TOB-HT product. The range of individual readings was 4.1–41.2 p.p.m. while smoking the 'usual brand' and 6.1–70.6 p.p.m. while smoking the TOB-HT. The sample day-to-day pooled standard deviation for expired breath CO was 4.7 p.p.m. for the 'usual brand' and 5.3 p.p.m. for the TOB-HT product.

Expired breath CO and %COHb are highly correlated. The linear relationship between blood measurements of %COHb and expired breath CO measurements is displayed in Figure 3. For the 14 subjects when smoking their 'usual brand', the R^2 for COHb versus expired breath CO is 0.84. The R^2 for %COHb versus expired breath CO when smoking the TOB-HT cigarette is 0.89. Both of these relationships are statistically significant. There is no statistically significant difference between the regression lines shown in Figure 3.

An additional experimental reading was obtained at a later time and is plotted with the original data in Figure 4. The data show reasonable agreement demonstrating that the %COHb determinations have a good long-term consistency, i.e., 25 day time-lapse between readings.

Discussion

An examination of the literature shows that the 8.2% group mean COHb value measured in this study after smoking the 'usual brand' is similar to COHb values previously reported by several other groups. Specifically, the following studies reported values comparable with our value of 8.2%: Butt *et al.*,[12] Sansores *et al.*,[13] Goldsmith *et al.*,[14] Haynes,[15] Ayres *et al.*,[16] Madany *et al.*,[17] Anderson *et al.*,[18] Lightfoot,[2] and Castledon and Cole.[19] These studies examined more than 181 subjects (some studies did not report the number of subjects). In nine other studies, the reported mean %COHb value was within a standard deviation (approximately 1–3 COHb units depending on the study) of 8.2%.

There were two primary findings provided by this study: (1) When measured over a 4 day period, carboxyhemoglobin and expired breath CO levels taken at the same time in the afternoon for each subject were consistently reproducible in a group of 14 subjects after smoking both their 'usual brand' (pooled standard deviation of 0.94% COHb) and a TOB-HT cigarette (pooled standard deviation of 1.37% COHb). An additional time point taken

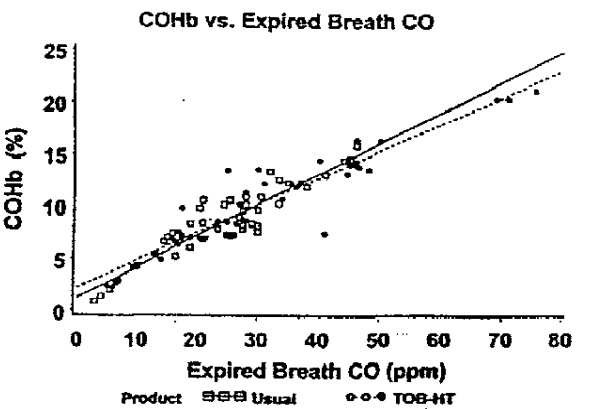

Figure 3 Graph showing the relationship between blood %COHb measurements and expired breath CO measurements

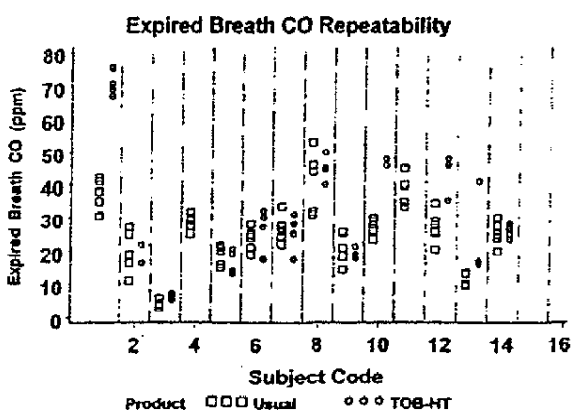

Figure 2 Graph of the repeatability of expired breath CO measurements for each subject when smoking either their 'usual brand' or the TOB-HT cigarette. Five measurements were taken

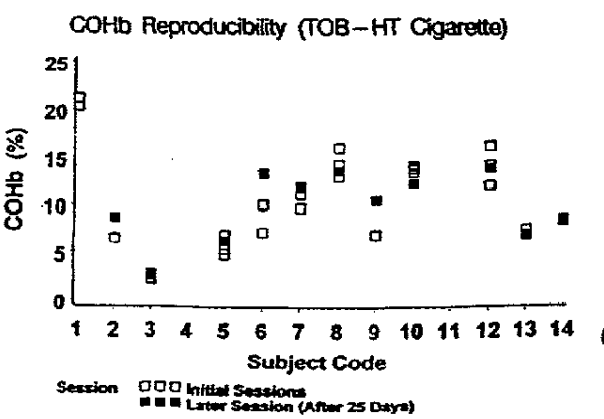

Figure 4 Graph of the reproducibility of %COHb measurements for each subject when smoking either their 'usual brand' or the TOB-HT cigarette. The measurements in the initial sessions were taken over 5 days. The later session measurement was taken 25

25 days later, was also similar to the three values taken during the 4 day period. (2) Carboxyhemoglobin and expired breath CO levels were elevated by 24.4 and 30.6%, respectively, after switching to the TOB-HT cigarette. This increase was not due to an increase in the number of cigarettes consumed since the subjects smoked an average of 21 cigarettes prior to the measurement session when smoking either their 'usual brand' or the TOB-HT cigarette.

The variability between the daily %COHb measurements might be expected to result primarily from daily differences in the number of cigarettes smoked prior to testing. However, using output from the Coburn, Forster, Kane model (CFK model) for predicting COHb levels,[8] it is estimated by assuming equal time spacing between cigarettes that irrespective of the number of cigarettes smoked per day, a smoker is predicted to be at approximately 85% of his peak COHb level after eight hours of a 16 h smoking day.

The following mathematical simulation uses the CFK model to illustrate that the magnitudes of the pooled day-to-day standard deviations of the %COHb measurements determined in this study are in the expected range. This simulation is based on several assumptions: (1) the time of day that the %COHb measurement is taken will vary over a 1 h range from target in either direction, specifically that the time from starting smoking to measurement is from 7 to 9 h with a target of 8 h; (2) the total number of cigarettes smoked per day varies about 15% in either direction from the normal number of cigarettes, i.e., the number of cigarettes per day is uniformly distributed from 85% of normal to 115% of normal. At the normal level of 35 total cigarettes per day, the %COHb standard deviation is predicted from the CFK model to be about 1.1%. This value is very close to the pooled day-to-day standard deviation value of 0.94 seen among the 'usual brand' smokers who had an average reported daily consumption of 34 cigarettes. A similar simulation showed that the variation in predicted %COHb is affected very little by the variation in the 'number of hours since initiating smoking' prior to the %COHb measurement. It should be noted that given its assumptions this procedure will slightly overstate the variability in the %COHb measurement because our usage of the CFK model assumes a steady-state exposure condition. In actual fact, if a smoker abruptly changed their smoking behavior, the %COHb level would only gradually approach a new steady-state level.

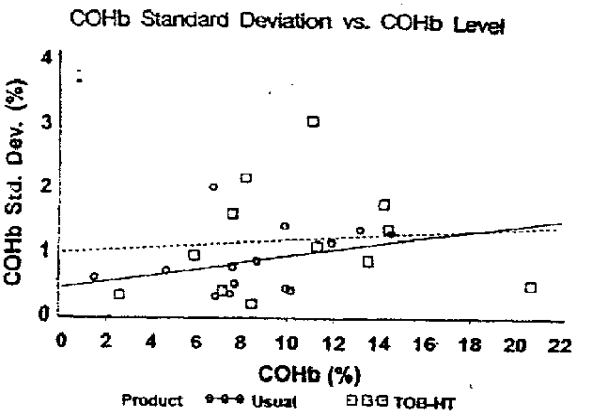

Figure 5 Graph of the standard deviation for the %COHb measurement *versus* the %COHb measurement itself

Using the above assumptions, the day-to-day standard deviation of individuals is predicted to increase approximately linearly with the number of cigarettes smoked per day. This result suggests that analyses which assume a constant variance from subject to subject should use a logarithmic transformation of the COHb values. However, this theoretical finding is subject to empirical verification since, e.g., light smokers might show a larger percentage variation in the number of cigarettes smoked per day than heavier smokers. A plot of the day-to-day variability for each subject *vs* the subject's mean value is given in Figure 5. This figure lends some support to the idea that there is a larger standard deviation at larger %COHb readings, but not as large a standard deviation as the model predicts assuming constant percent variation in smoking behavior. If the %COHb levels of the smokers span a wide range, it may be necessary to transform the data to achieve homoscedasticity, i.e., similar variance among data sets. However, a logarithmic transformation may be too dramatic. Whether a square root or some other transformation would work better will be explored further as more data become available. With the data obtained thus far, the protocol used in this study appears to provide a repeatable and useful method for the determination of %COHb levels in smokers.

References

1 Hanson HB, Hastings AB. The effect of smoking on the carbon monoxide content of blood. *Tuberculosis-Kereszturi et al.* 1933; **100(19)**: *1481–1484.*

2 Lightfoot NF. Chronic carbon monoxide exposure. *Proceedings of the Royal Society of Medicine.* 1972; **65**: 798–799.

3 Klesges RC, Klesges LM. The relationship between body mass and cigarette smoking using a biochemical index of smoking exposure. *International Journal of Obesity.* 1993; **17**: 585–591.
4 Wyman J *et al.* Examination of Haldane's first law for the partition of CO to O_2 to hemoglobin Ao. *Biopolymers.* 1982; **21**: 1735–1747.
5 Smith RP. Toxic responses of the blood. Chapter 8 in *Toxicology–The Basic Science of Poisons,* fourth edition, eds. Klaassen CD, Amdur MO, Doull J. McGraw-Hill, NewYork, 1993, pp. 268.
6 Peterson JE, Stewart RD. Absorption and elimination of carbon monoxide by inactive young men. *Archives of Environmental Health.* 1970; **21**: 165–171.
7 Amdur MO. Air pollutants. Chapter 25 in *Toxicology–The Basic Science of Poisons,* fourth edition, eds. Klaassen CD, Amdur MO, Doull J. McGraw-Hill, New York, 1993, pp. 854–871.
8 Coburn Rf, Forster RE, Kane PB. Considerations of the physiological variables that determine the blood carboxyhemoglobin concentration in man. *Journal of Clinical Investigation.* 1965; **44**: 1899–1910.
9 Ingebrethsen BJ *et al.* A numerical model of carboxyhemoglobin concentration during smoking. Abstract 60, 50th Tobacco Chemists' Research Conference, October 23, 1996.
10 Sutherland G, Russell MAH, Stapleton JA, Feyerabend C. Glycerol particle cigarettes: a less harmful option for chronic smokers. *Thorax.* 1993; **48**: 385–387.
11 Nurses Ready Reference™. Diagnostic Tests. Spring House 1991; pp. 250.
12 Butt J, Davies M, Jones JG, Sinclair A. Carboxyhemoglobin levels in blast furnace workers. *Annals of Occupational Hygiene.* 1974; **17**: 57–63.
13 Sansores RH, Pare PD, Abboud RT. Acute effect of cigarette smoking on the carbon monoxide diffusing capacity of the lung. *American Review of Respiratory Disease.* 1992; **146**: 951–958.
14 Goldsmith JR, Terzaghi, Hackney JD. Evaluation of fluctuating carbon monoxide exposures. *Archives of Environmental Health.* 1963; **7**: 647–663.
15 Haynes RL. Carbon monoxide poisoning from non-tobacco cigarettes. *Journal of MAG.* 1983; **72**: 553–555.
16 Ayres SM, Evans RG, Buehler ME. The prevalence of carboxyhemoglobinemia in New Yorkers and its effects on the coronary and systemic circulation. *Preventive Medicine.* 1979; **8**: 323–332.
17 Madany IM *et al.*, Occupational exposure to carbon monoxide during charcoal meat grilling. *The Science of the Total Environment.* 1992; **114**; 141–147.
18 Anderson WH, Rivera C, Bright M. Carboxyhemoglobin blood levels after smoking one cigarette in relation to puff profile. 1975 Symposium Nicotine and Carbon Monoxide, presented by the Tobacco & Health Research Institute, and The Kentucky Tobacco Res. Board, Nov. 17, 18, 1975, University of Kentucky, Lexington, Kentucky.
19 Castleden CM, Cole PV. Variations in carboxyhaemoglobin levels in smokers. *British Medical Journal.* Dec. 28, 1974; 736–738.